

and theoretical perspectives. A recent modification proved successful in reducing the number of Trp anchors to one near each end of the peptide. The resulting GWALP23 (acetyl-GGALW⁵(LA)₆LW¹⁹LAGA-[ethanol]amide) displays greater sensitivity to lipid-peptide hydrophobic mismatch. Now we further modify GWALP23 to incorporate a single tyrosine, replacing W⁵ with Y⁵. The resulting peptide, Y⁵GWALP23 (acetyl-GGALY⁵(-LA)₆LW¹⁹LAGA-amide) has now only a single remaining Trp residue which is accessible for fluorescence experiments. By labeling specific alanines in Y⁵GWALP23 with deuterium, we were able to use solid-state ²H-NMR spectroscopy to examine the peptide orientation in hydrated lipid bilayer membranes. The peptide orients well in membranes. The substitution of Y⁵ for W⁵ has remarkably little influence upon the GWALP23 apparent tilt or dynamics in bilayer membranes of DOPC, DMPC or DLPC. A second analogue with double Tyr anchors, Y^{4,5}GWALP23, was found to be generally less responsive to the bilayer thickness and to exhibit lower apparent tilt angles and possibly more extensive dynamics. Indeed the case with multiple Tyr anchors seems to be quite similar to the situation when multiple Trp anchors are present, as in the original WALP model peptides.

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Comparison of Proline Substitutions at Positions 8 and 10 in WALP19

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Proline is often found in the transmembrane helices of naturally occurring membrane-spanning proteins. It is thought that proline introduces a kink and disrupts the helical structure, possibly causing an overall loosening of the helix, forming a "molecular hinge." The influence of proline at the center of the model peptide WALP19-P10 (acetyl-GWWLALALAP¹⁰ALALALWWA-ethanolamide) has been characterized in several lipid bilayer membranes (see *Biochemistry* 48, 11883). In this research, we employ solution NMR spectroscopy to examine the structures of WALP19-P8 (acetyl-GWWLALAP⁸ALALALWWA-ethanolamide) as well as WALP19-P10 in SDS micelles. The spectral quality is better for WALP19-P8 than for the proline-10 peptide. Based upon >135 restricting NOE assignments throughout the sequence (including interactions that link residues L6 and A9, as well as A7 and L10, on either side of the proline), we have determined a kinked structure for WALP19-P8. Preliminary analysis suggests that some of the intrahelix hydrogen bonding is disrupted both near and far from the proline, indeed resulting in a general loosening of the helical structure. The spectra for WALP19-P10 are more difficult to interpret structurally, despite a high number of assigned NOE restraints (>220). Local dynamics near the hinge region and sparse NOE connectivity in the vicinity of proline permit multiple solutions for the WALP19-P10 backbone geometry. Conversely, local bond dynamics appear to be a minor factor in WALP19-P8. The interplay among proline location, spectral quality, and structure determination will be discussed.

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Influence of Glycine Substitutions on Designed Proline-Containing Transmembrane Peptides

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Membrane-spanning WALP peptides of general sequence acetyl-GWW(LA)_nLWWA-amide, in which "n" may range from about 3 to 12, have been well characterized. A kink induced by a central proline residue in WALP19-P10 (acetyl-GWWLALALAP¹⁰ALALALWWA-ethanolamide) has also been characterized (see *Biochemistry* 48: 11883). It was furthermore observed that the ²H-quadrupolar splittings from labeled alanine side chains in WALP19-P10 were especially sensitive to Leu-to-Ala substitutions elsewhere in the sequence. In this work, we examined the consequences of Leu-to-Gly substitutions within the proline-containing WALP19-P10, from a perspective that Leu-to-Gly substitutions could reasonably be expected to produce larger consequences than Leu-to-Ala substitutions. Circular dichroism spectra indicate small reductions in helicity for Gly substitutions in WALP19-P10, with the mean residue ellipticity values being similar for Leu-to-Gly substitutions on either side of the proline. Using ²H-alanines as probes within the sequence, we have found from deuterium NMR spectroscopy of peptides incorporated within oriented lipid bilayer samples that the segment N-terminal to proline is generally more susceptible to Leu-to-Gly substitutions on both sides of the proline. In contrast, the C-terminal domain is largely unaffected by the glycine substitutions in either the N- or C-terminal

segment. Substitutions near the C-terminus have little effect on either segment.

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Helix-Helix Interactions in Phospholipid Model Membranes as a Function of Acyl Chain Unsaturation

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Previous work in our laboratory has identified lipid-protein interactions that appear unique to the class of lipids having polyunsaturated fatty acids (PUFA). In this work we extend these studies to examine the effect of PUFA on lipid mediated protein-protein interactions. Specifically, we have used molecular dynamics (MD) computer simulation methods to compute the potential of mean force (PMF) between a pair of alpha helical peptides, i.e. the free energy as a function of separation. Constant pressure, constant temperature simulations of the identical peptides in phosphidylcholine bilayers with saturated chains (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) and those containing PUFA (1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine) have been carried out using the techniques of umbrella sampling and the weighted histogram analysis method. These lipids were carefully chosen for their near identical hydrophobic thicknesses so that the effect of unsaturation could be uniquely examined.

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RIP Pulchellin Isoforms: Biomembrane Models Showing Different Intoxication Mechanisms Between Them

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Pulchellin, a type-2 Ribosome Inactivating Protein (RIP), whose action mechanism proposed would be initiate by B-chain binding on glycoconjugate onto the cell surface (lectinic chain). Into the cell, the A-chain (toxic chain) binding in ER lipids and stopping protein synthesis at ribosome inducing cell death. Was showed that Puchelin isoforms have different toxicity and were separated subgroups P1, PII, PIII, PIV based: toxicity, secondary structure, specificity for sugar binding. PII and PIV subgroups present structural identity above 70%, however PII is five-fold more toxic than PIV (Castilho, et al. *FEBS journal*, 2008). The present studies show results of interaction between model membranes and the two isoforms (PII and PIV) using Langmuir monolayers (LM) and giant unilamellar vesicles (GUVs). PII and PIV bond to LM-DPPC in the same extension, but when monolayers contented 5% of GalCer, PII remained strongly interacting with the mixed monolayer and PIV seems to be inhibited by the presence of the glycolipids. In GUVs-POPC, both PII and PIV destroyed the vesicles, however, in GUVs-POPC+5%GalCer, only PII induced vesicles decreased in their surface around 30% and some invaginations could be observed. After 20 minutes, small vesicles were observed inside of the GUV structure. For PIV, instead of invaginations, only fluctuations on the GUV surface were verified. Increasing PIV concentration the GUVs were destruction. In view of the results, one can conclude that the key for understanding differences in pulchellin isoforms toxicity is the interaction between B-chain lectinic and the glycosyl residues on cell surface.

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A Putative Role for Lipid-Protein Interactions in the Localisation of Glycosyltransferases within the Cell?

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Fukutin-I is a member of a family of putative O-linked glycosyltransferases involved in the glycosylation of the dystrophin complex. Mutations in this family of proteins have been linked to number of congenital muscular dystrophies and are characterised by the hypoglycosylation of the alpha-dystroglycan. The function of these proteins relies critically on their location within the endoplasmic reticulum (ER)/Golgi Apparatus (GA). The retention of proteins in these compartments is thought to be mediated by the interaction of the N-terminal transmembrane domain with the atypical lipid composition of these compartments although the molecular basis for this retention is far from clear. To address this question, we are currently studying how the composition of the lipid bilayer within the ER/GA modifies the structure, oligomeric state and lateral segregation of these proteins. Preliminary studies have established an expression system for the production and labelling of the transmembrane domains of these proteins. Using a combination of liquid and solid-state NMR we are characterising how the structure of the N-terminal transmembrane domain of Fukutin-I varies as a function of lipid/detergent composition. Solid-state NMR methods are revealing how the lipid bilayer composition changes the dynamics of the protein within the bilayer thus providing insights into the proteins oligomeric state. In conjunction with cross-linking and fluorescence resonance